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Title: Parasternal intercostal muscle activity during methacholine-induced bronchoconstriction

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Running Title: Respiratory muscle EMG in methacholine challenge

Abstract: Neural respiratory drive, measured via the parasternal intercostal electromyogram (EMGpara), provides a non-invasive index of the load-capacity balance of the respiratory muscle pump. Previous studies in patients with obstructive lung disease have shown strong relationships between EMGpara and the extent of both airflow obstruction and hyperinflation. The relative influence of the two factors has not, however, been described. Airflow obstruction was induced via methacholine challenge testing in twenty-five adult humans. Forced expiratory volume in one second (FEV1) and surface EMGpara during tidal breathing were measured following each dose, with twenty of the participants also undergoing measurements of inspiratory capacity (IC) at each stage. Linear mixed model (LMM) analysis was used to assess dose-wise changes in FEV1 and EMGpara, and thereafter to determine the

influence of changes in FEV1 and IC on change in EMGpara. Median (IQR) FEV1 decreased significantly (96.00 (80.00 - 122.30)%predicted to 67.80 (37.98 - 92.27)%predicted, p<0.0001) and EMGpara increased significantly (5.37 (2.25 - 8.92)μV to 6.27 (3.37 - 19.60)μV, p<0.0001) from baseline to end of test. LMM analysis showed a significant interaction between methacholine dose and induced change in EMGpara, with an increase in EMGpara of 0.24 (95% confidence interval 0.11 - 0.37)μV per methacholine dose2. Change in FEV1 further influenced this relationship (increase in slope of 0.002 (0.004 - 0.001)μV dose-2 per %predicted fall in FEV1, p=0.011), but not with change in IC. These data suggest that bronchoconstriction exerts a more potent influence on levels of EMGpara than changes in end-expiratory lung volume during methacholine challenge.

New Findings: The parasternal intercostal electromyogram (EMGpara) is known to provide an accurate, non-invasive index of respiratory load-capacity balance. Although relationships between EMGpara and both airflow obstruction and hyperinflation have been shown, the independent contribution of each factor has not been examined. Reductions in airway calibre and inspiratory capacity (IC) along with increases in EMGpara were induced via methacholine challenge. A strong inverse relationship was observed between EMGpara and airway obstruction with no influence of IC. These data suggest that EMGpara is more strongly influenced by airway calibre than by changes in end-expiratory lung volume during airway challenge testing.

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Parasternal intercostal muscle activity during methacholine-induced bronchoconstriction

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New findings

- *What is the central question of this study?*

The parasternal intercostal electromyogram (EMGpara) is known to provide an accurate, non-invasive index of respiratory load-capacity balance. Although relationships between EMGpara and both airflow obstruction and hyperinflation have been shown, the independent contribution of each factor has not been examined.

- *What is the main finding and its importance?*

Reductions in airway calibre and inspiratory capacity (IC) along with increases in EMGpara were induced via methacholine challenge. A strong inverse relationship was observed between EMGpara and airway obstruction with no influence of IC. These data suggest that EMGpara is more strongly influenced by airway calibre than by changes in end-expiratory lung volume during airway challenge testing.

Abstract

Neural respiratory drive, measured via the parasternal intercostal electromyogram (EMGpara), provides a non-invasive index of the load-capacity balance of the respiratory muscle pump. Previous studies in patients with obstructive lung disease have shown strong relationships between EMGpara and the extent of both airflow obstruction and hyperinflation. The relative influence of the two factors has not, however, been described. Airflow obstruction was induced via methacholine challenge testing in twenty-five adult humans. Forced expiratory volume in one second (FEV_1) and surface EMGpara during tidal breathing were measured following each dose, with twenty of the participants also undergoing measurements of inspiratory capacity (IC) at each stage. Linear mixed model (LMM) analysis was used to assess dose-wise changes in FEV_1 and EMGpara, and thereafter to determine the influence of changes in FEV_1 and IC on change in EMGpara. Median (IQR) FEV_1 decreased significantly (96.00 (80.00 – 122.30)%predicted to 67.80 (37.98 – 92.27)%predicted, $p<0.0001$) and EMGpara increased significantly (5.37 (2.25 – 8.92)μV to 6.27 (3.37 – 19.60)μV, $p<0.0001$) from baseline to end of test. LMM analysis showed a significant interaction between methacholine dose and induced change in EMGpara, with an increase in EMGpara of 0.24 (95% confidence interval 0.11 – 0.37)μV per methacholine dose^2. Change in FEV_1 further influenced this relationship (increase in slope of 0.002 (0.004 – 0.001)μV dose^{-2} per %predicted fall in FEV_1, $p=0.011$), but not with change in IC. These data suggest that bronchoconstriction exerts a more potent influence on levels of EMGpara than changes in end expiratory lung volume during methacholine challenge.

Abstract word count: 245 words

Abbreviations

ECG, electrocardiogram; EMGpara, parasternal intercostal electromyogram; EOT, end of test; FEV_1, forced expiratory volume in one second; FRC, functional residual capacity; FVC, forced vital capacity; Hz, Hertz; IC, inspiratory capacity; IQR, interquartile range; kg, kiligram; L, litre; LMM, linear mixed models; mg, milligram; µg, migrogram; µV, microvolt; min, minute; ml, millilitre; mm, millimetre; ms, millisecond; NRD, neural respiratory drive; PC20, provocative concentration of methacholine inducing a 20% fall in FEV_1; REML, restricted maximum likelihood; RMS, root mean square; sec, second; TLC, total lung capacity.

Introduction

Measures that allow the severity of respiratory disease to be accurately quantified are essential for optimal patient management. The majority of assessment techniques in current use examine a single component of respiratory function, such as airflow obstruction or lung volumes. Measurements of neural respiratory drive (NRD), obtained from respiratory muscle electromyography, provides an index of the overall load on the respiratory system and represents a promising method for assessment of the severity of lung disease.

The pathophysiological changes that occur in obstructive lung diseases adversely influence the load-capacity balance of the respiratory muscle pump. Elevated airway resistance, together with distal airway collapse and gas trapping resulting in intrinsic positive end-expiratory pressure, all impose loads on the respiratory muscles during inspiration. Additionally, increases in end-expiratory lung volume result in respiratory muscle shortening and mechanical disadvantage, reducing their force-generating capacity and leading to functional weakness (Moxham & Jolley, 2009). In the face of such changes in load and capacity, compensatory increases in NRD occur to maintain ventilation at an appropriate level for blood gas homeostasis.

Studies in patients with moderate to severe lung disease have demonstrated strong relationships between NRD and the extent of both airflow obstruction and hyperinflation (Jolley *et al.*, 2009; Reilly *et al.*, 2011). The relative influence of the two factors has not, however, been described. Reilly *et al.* (2011) reported progressive increases in NRD, measured using both the diaphragm (EMGdi) and

parasternal intercostal electromyogram (EMGpara), during incremental exercise in patients with cystic fibrosis and matched healthy controls. The patients demonstrated exercise-induced dynamic hyperinflation, but the relative influence of such changes in end expiratory lung volume over and above that imposed by airway resistance and the metabolic load of the exercise was not described.

Airway challenge testing allows stepwise decrements in pulmonary function to be induced under controlled circumstances. Bronchoconstriction is the primary change occurring during airway challenge testing, although hyperinflation may also occur in some individuals (American Thoracic Society, 2000). Airway challenge testing provides a model by which the changes in NRD resulting from acute, stepwise changes in pulmonary function can be assessed, without the confounding influences that may be encountered in a longitudinal study in patients with lung disease such as the metabolic influences of infection or changes in medication.

The aims of this study were, therefore, to investigate the changes in EMGpara induced by airway challenge testing, and to determine the relative independent contributions of bronchoconstriction and increased end-expiratory lung volume. We hypothesised that EMGpara would increase in relation to the degree of bronchoconstriction, and that hyperinflation would exert a further influence on changes in EMGpara.

Methods

Ethical approval for the study was granted by the King's College Hospital Research Ethics Committee (reference number 09/H0808/100). All subjects gave written informed consent for participation in the study. All ethical aspects of the experiments conformed to the Declaration of Helsinki.

Subjects

Thirty two subjects with a self reported history of atopy, asthma or known bronchial hyperresponsiveness were studied. The median (IQR) age of the group was 29.2 (24.3 – 33.5) years. All subjects had spirometry within normal limits at baseline.

Anthropometry

Height was measured using a wall-mounted stadiometer (Harpenden, Holtain Ltd, Crymych, UK) with a resolution of 1mm (range 600-1200 mm). The subject was asked to remove footwear and stand upright with heels, back and occiput against the stadiometer. A 1kg weight was placed on the stadiometer carriage to improve accuracy.

Weight was measured using an electronic medical scale (HR Person Scale, Marsden Ltd, Henley on Thames, UK) with a resolution of 0.05kg and a maximum weighing capacity of 300kg. The subject was asked to remove footwear and any heavy clothing and remain stationary on the scale until a steady reading was obtained.

Parasternal intercostal electromyography

Skin was prepared in accordance with international guidelines for EMG recording (Hermans *et al.*, 2000). Initially the skin was scrubbed with an abrasive gel (Nuprep, Weaver and Company, Aurora, USA) to remove dead skin cells and excessive sebum in order to minimise electrode-skin impedance. Any remaining gel and sebum was then removed with an alcohol-impregnated wipe before self-adhesive silver-silver-chloride electrodes were applied (Kendall Arbo, Tyco Healthcare, Neustadt/Donau, Germany). Electrodes were applied in the second intercostal space bilaterally, 3cm lateral to the sternum, with a reference electrode placed on the acromion process of the scapula.

Signals were amplified and band-pass filtered between 10 and 3,000Hz, with an additional analogue notch filter at 50Hz to minimise mains frequency interference, using a PClab 3808 Biomedical Amplifier (Yinghui Medical Technology Co. Ltd, Guangzhou, China). EMG signals were acquired (PowerLab 16SP, ADInstruments, Sydney, Australia) and displayed on a laptop computer (MacBook Pro, Apple, Cupertino, USA) running LabChart software (Version 7.2, ADInstruments Pty, Colorado Springs, USA) with analogue to digital sampling at 10kHz. Both amplifier and analogue-to-digital convertor were earthed to suitable points in the laboratory. An additional 20Hz post-acquisition digital high-pass filter was applied via the LabChart software to assist in the removal of ECG artefact. Data were displayed as both raw EMG and as a root mean square (RMS) trace, using a moving average window of 50ms (Ives & Wigglesworth, 2003).

EMGpara was recorded during tidal breathing. Subjects were seated upright in a chair with back supported, arms placed on armrests and feet flat on the floor to minimise trunk postural activity. Subjects were instructed to remain still and not to speak throughout the recording period.

EMGpara activity, occurring between QRS complexes, was highlighted and the peak RMS EMGpara calculated. The mean peak RMS EMGpara per breath was calculated over the final minute of each recording period.

Spirometry

Spirometry was performed in accordance with American Thoracic Society/European Respiratory Society (ATS/ERS) criteria (Miller *et al.*, 2005) using a portable electronic spirometer (KoKo spirometer, Ferraris Respiratory, Louisville, CO, USA). The correct technique was explained and demonstrated to each subject prior to testing. The subject was seated upright with head and neck in a neutral or slightly extended position, fitted with a noseclip and asked to seal their lips tightly around a flanged mouthpiece. The subject was then asked to inspire rapidly and fully to total lung capacity (TLC) and then, following a pause of no more than one second, to perform a maximal forceful exhalation and to maintain expiratory flow until no more air could be expelled. Subjects were encouraged to "blast", rather than "blow", the air from the lungs, and strong verbal encouragement was given throughout each manoeuvre to promote complete exhalation. Any traces showing evidence of incomplete exhalation, glottic closure, cough or air leak were rejected. Repeated efforts were

performed at baseline until three acceptable manoeuvres were obtained. Acceptable repeatability was considered to have been obtained when the difference between the largest and second-largest values was less than 150ml or 5%. Values for forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) were expressed as a percentage of predicted values (Quanjer *et al.*, 1993).

Methacholine challenge testing

The methacholine challenge test was performed in accordance with international guidelines (American Thoracic Society, 2000). Testing was performed in a well-ventilated room. Subjects were seated in a chair suitable for EMG recording and baseline pulmonary function testing was performed as described.

The methacholine challenge was performed using a dosimeter (KoKo USB digidoser, Ferraris Respiratory, Louisville, CO, USA) and nebuliser (DeVilbiss 646 characterised nebuliser, Ferraris Respiratory, Louisville, CO, USA) with a certified output of 1.134ml min^{-1}. Subjects were required to wear a nose clip and inhale and exhale through the mouthpiece at a maximum flow of 0.5L sec^{-1} imposed by a flow restrictor. Use of a dosimeter and flow restrictor improve accuracy and repeatability of the dose delivered (American Thoracic Society, 2000). Inspiratory and expiratory duration were also fixed at five seconds using auditory prompts. Subjects were given verbal encouragement to maintain respiratory flow at the required rate and for a sufficient duration.

Measurement of respiratory flow and volume

Respiratory flow was measured using a pneumotachograph (range 0-800L min^{-1}, model 4813, Hans Rudolph Inc. Kansas City, USA), attached to a flanged mouthpiece with the subject wearing a noseclip. Differential pressure across the pneumotachograph was measured using a pressure transducer (MP45-16, Validyne, Northridge, USA). The signal from the transducer was amplified (CD280, Validyne, Northridge, USA), and recorded and displayed alongside EMGpara by the acquisition software (LabChart) with analogue-to-digital sampling of 100Hz (PowerLab 16SP). Volume was measured via digital integration of the flow signal using LabChart software.

Inspiratory capacity (IC) was obtained by asking the subject to perform a slow maximal inhalation to TLC from functional residual capacity (FRC). This was repeated three times, each effort interspersed with three to five tidal breaths, and the mean of the three efforts recorded. Significant hyperinflation was defined as a decrease in IC of ≥300ml (Lougheed *et al.*, 2006).

Protocol

All testing was performed on a single occasion. Participants were requested to refrain from taking short-acting beta-2 agonists or antihistamine medication and from consuming products containing caffeine (coffee, tea, cola, chocolate) on the day of testing. Subjects were familiarised with study instrumentation and the protocol explained in detail. Anthropometric and demographic data were recorded

and baseline spirometry performed. The five-breath dosimeter protocol was employed, with an initial diluent step using 0.9% saline followed by methacholine concentrations of 0.0625, 0.25, 1, 4, 8, 16 and 32 mg ml^{-1} (Acetyl-B-Methyl Choline, Nova Laboratories, Wigston, Leicestershire, UK). Doses were delivered at five minute intervals. At the beginning of testing, the diluent was administered via the digidoser and a single technically acceptable FEV_1 manoeuvre performed at 30 and 90 seconds following dose delivery and the lower of the two values used. Following the second forced expiratory manoeuvre, three IC manoeuvres were performed and EMGpara was then recorded during tidal breathing for the remaining three minutes until delivery of the next dose. The protocol was repeated, using increasing doses of methacholine, until the subject's FEV_1 had fallen by 20% or more from the post-diluent value, or until the maximum methacholine dose had been delivered. If a 20% or greater fall in FEV_1 was elicited, the test was discontinued and bronchoconstriction reversed through the administration of 400µg salbutamol.

PC20 is defined as the provocative concentration of methacholine inducing a 20% fall in FEV_1, and was calculated using the formula:

$$\text{PC20} = anti\log\left[\log C_1 + \frac{(\log C_2 - \log C_1)(20 - R_1)}{R_2 - R_1}\right]$$

Where:

C_1 = penultimate methacholine concentration

C_2 = final methacholine concentration

R_1 = percent fall from post-diluent stage in FEV_1 after C_1

R_2 = percent fall from post-diluent stage in FEV_1 after C_2

Statistical analysis

Differences in EMGpara, FEV_1 and IC from baseline (measurements made post-diluent step) to end of test (EOT) were assessed using Wilcoxon's matched pairs test. Differences between the groups of subjects in whom significant hyperinflation was and was not observed were assessed using the Mann-Whitney test. Linear mixed model (LMM) analysis was then used to analyse dose-wise changes in both FEV_1 and EMGpara, and thereafter to determine the independent influences of changes in FEV_1 and IC on change in EMGpara. Models were also fitted to determine the trajectories of EMGpara between the hyperinflating and non-hyperinflating groups. LMM is an extension of multiple regression, allowing irregularly spaced serial data for individuals to be combined in a single linear regression model, providing estimates of both longitudinal individual changes and group patterns (West, 2009).

Fixed effects in the model were methacholine dose and FEV_1 when examining the entire cohort, with further models constructed incorporating IC both as a continuous variable and a binary term (change in IC greater or less than 300ml). Random effect variables in all models comprised methacholine dose and an intercept term.

Models were estimated using restricted maximum likelihood (REML), with all models centred on the diluent dose such that the intercept terms would be interpretable as the mean value of the dependent variable at commencement of testing, and slope would represent the change in the dependent variable per dose. Model fit to dose as linear and quadratic functions was evaluated due to the quadrupling/doubling

dose protocol used and the known sigmoidal shape of the conventional FEV_1 dose-response curve (American Thoracic Society, 2000). The selection of an appropriate covariance structure for the residuals and the validity of the quadratic versus linear functions of methacholine dose were assessed by likelihood ratio testing based on the -2 REML log-likelihood difference between the original and modified models (West, 2009).

Results

Participant characteristics are shown in Table 1. All 32 subjects underwent full methacholine challenge testing with measurements of spirometry and EMGpara. A subset of 20 individuals had additional measurement of inspiratory capacity (termed "IC sub-group").

	Entire cohort (n=32)	IC sub-group (n=20)
Age (years)	29.2 (24.3 – 33.5)	29.0 (25.1 – 34.1)
Sex (male : female)	12 : 20	6 : 14
Height (m)	1.70 (1.63 – 1.76)	1.71 (1.66 – 1.76)
Weight (kg)	68.4 (59.9 – 77.8)	68.4 (63.1 – 77.6)
BMI (kg m^{-2})	24.4 (21.8 – 25.2)	24.1 (21.5 – 25.0)
Ethnicity (n (%))	White: 26 (81.3%) Black: 1 (3.1%) Asian: 4 (12.5%) Other: 1 (3.1%)	White: 19 (95%) Black: 1 (5%)
Baseline FEV_1 (%predicted)	97.1 (88.9 – 102.2)	96.9 (86.3 – 106.9)

Table 1 Characteristics of subjects undergoing methacholine challenge testing, with measurement of FEV_1 and EMGpara only (entire cohort), and additional measurement of inspiratory capacity (IC sub-group)

Of the 32 participants, 25 exhibited a clinically significant response to methacholine, defined as a decrease of 20% or greater in FEV_1. The median (IQR) change in FEV_1 was 27.8 (35.6 – 22.7)% in the responder subset, and 7.2 (14.1 – 4.1)% in the non-responder group. All 20 of the subjects in the IC sub-group showed a clinically significant response, with a median (range) fall in FEV_1 of 28.9 (37.0 – 22.8)%.

Significant changes were observed from baseline to end of test (EOT) in FEV_1 and EMGpara in both the entire cohort and the 'responder' subset. The change in FEV_1 was significant in the non-responder group but not the change in EMGpara. Significant changes were observed in all parameters (FEV_1, EMGpara and IC) in the IC sub-group of subjects. These results are summarised in Figures 1, 2 and 3 and Table 2.

		Baseline	End of test	p value
Entire cohort (n=32)	FEV_1 (% predicted)	97.10 (80.00 – 122.30)	70.95 (37.98 – 100.70)	<0.0001
	EMGpara (μV)	5.02 (2.00 – 8.92)	5.86 (2.37 – 19.60)	<0.0001
Responders (n=25)	FEV_1 (% predicted)	96.00 (80.00 – 122.30)	67.80 (37.98 – 92.27)	<0.0001
	EMGpara (μV)	5.37 (2.25 – 8.92)	6.27 (3.37 – 19.60)	<0.0001
Non-responders (n=7)	FEV_1 (% predicted)	98.46 (90.67 – 103.04)	91.43 (82.75 – 98.86)	0.028
	EMGpara (μV)	3.54 (3.15 – 3.91)	4.59 (4.35 – 5.88)	0.091
IC measurement sub-group (n=20)	FEV_1 (% predicted)	96.86 (80.00 – 122.30)	67.50 (37.98 – 92.27)	<0.0001
	EMGpara (μV)	5.37 (2.85 – 8.92)	6.63 (3.77 – 19.60)	0.0012
	IC (% predicted)	91.9 (65.6 – 133.3)	84.9 (39.8 – 129.0)	0.0037

Table 2 Baseline to end-of-test changes in FEV_1, EMGpara and IC in the full study cohort and subgroups.

LMM analysis demonstrated a significant positive interaction between a quadratic function for methacholine dose and change in EMGpara. There was an additional effect of the change in FEV_1 by dose on EMGpara, with the slope of the EMGpara versus $dose^2$ line increasing by 0.002µV $dose^{-2}$ (full cohort) or 0.003µV $dose^{-2}$ (Part B cohort) for each percent of predicted decrease in FEV_1. See Table 3 for full details of slopes and intercepts of these interactions. There was no significant effect of FEV_1 on the intercept of the EMGpara versus dose relationship, indicating no significant influence of baseline FEV_1 on pre-challenge EMGpara. Figure 4 shows the LMM-predicted EMGpara values plotted against measured FEV_1.

	Covariate	Coefficient (95% CI)	p value
Entire cohort (n=32)	Dose number^2	For intercept: 4.09 (0.61 – 7.57)	0.22
		For slope: 0.24 (0.11 – 0.37)	<0.001
	FEV_1	For intercept: 0.01 (-0.03 – 0.04)	0.689
		For slope: -0.002 (-0.004 – -0.001)	0.01
IC sub-group (n=20)	Dose number^2	For intercept: 4.21 (0.34 – 8.07)	0.033
		For slope: 0.27 (0.12 – 0.42)	0.001
	FEV_1	For intercept: 0.01 (-0.03 – 0.05)	0.537
		For slope: -0.003 (-0.005 – -0.001)	0.011

Table 3 Linear mixed models for covariates in the full study cohort and those undergoing measurement of IC. Intercepts are in μV, slopes in μV per dose number^2 (as dose was treated as a quadratic function).

Of the 20 participants undergoing measurement of IC, 10 demonstrated significant hyperinflation, defined as a decrease in inspiratory capacity of greater than or equal to 300ml (Lougheed *et al.*, 2006). There were no differences in the characteristics of the subjects in the hyperinflating (HI) and non-hyperinflating (NH) groups, nor were there differences in the degree of bronchoconstriction or change in EMGpara at EOT between the HI and NH groups (Table 4).

	Hyperinflating group (n=10)	**Non-hyperinflating group (n=10)**	**p value**
ΔFEV_1 (%)	-32.5 (-36.2 – -27.5)	-23.3 (-38.3 – -22.5)	0.22
ΔEMGpara (%)	25.3 (-4.6 – 95.8)	40.1 (1.2 – 101.1)	0.78

Table 4 Relative percentage changes in FEV_1 and EMGpara in the subjects demonstrating a reduction in inspiratory capacity of ≥300ml (hyperinflating group) and those showing a change in IC of <300ml (non-hyperinflating group).

Inspection of the residuals from the models diagnostics derived from LMM analysis showed that the IC data did not satisfy the assumption of homoscedasticity. The model was therefore reassessed following logarithmic transformation of the IC values. LMM analysis did not demonstrate a significant effect of the change in IC by dose on change in EMGpara, either when treated as a continuous variable (Table 5) or as a binary outcome (change in IC of greater than or less than 300ml).

Covariate	Coefficient (95% CI)	p value
Dose number^2	For intercept: 4.19 (-7.71 – 16.09)	0.477
	For slope: 0.01 (-0.74 – 0.77)	0.972
FEV_1	For intercept: 0.01 (-0.03 – 0.06)	0.578
	For slope: -0.003 (-0.005 – -0.001)	0.006
logIC	For intercept: 0.02 (-2.62 – 2.66)	0.988
	For slope: 0.07 (-0.10 – 0.24)	0.438

Table 5 Linear mixed models for covariates in Part B. Intercepts are in µV, slopes in µV per dose number^2 (as dose was treated as a quadratic function). Values for the influence of dose^2 and FEV_1 on EMGpara are different to those displayed in Table 2 due to the incorporation of additional data into the model.

Discussion

This study has investigated in detail respiratory load compensation using the changes in parasternal intercostal muscle activity during chemical airway challenge, and has allowed the investigation of the interaction between decrements in key pulmonary function parameters and change in respiratory muscle activity. The hypothesis that EMGpara would increase in proportion with decrements in FEV_1 was supported; the secondary hypothesis, that hyperinflation would independently result in further increases in EMGpara, was refuted. These data suggest that during airway challenge testing bronchoconstriction imposes a greater relative load on the respiratory system as indicated by increased neural drive to the parasternal intercostal muscles than concomitant changes in end expiratory lung volume.

Maarsingh *et al.* (2002) examined the relationship between FEV_1 and respiratory muscle activity assessed using both parasternal intercostal and surface diaphragm EMG during histamine-induced bronchoconstriction in 15 asthmatic children. As with the current study, they observed a good agreement between the degree of airway narrowing and increases in EMG activity. Unlike the current study, however, the EMG data were expressed as the logarithm of the ratio of the EMG activity at each stage to that at baseline. This linear increase in the log transformed EMG data suggests that EMG activity in their study increased exponentially. Close agreement was also observed between the increase in EMG activity and decreases in FEV_1 expressed in 5% decrements from baseline, determined by linear interpolation. Expressing the data in this way however, risks biasing the results towards a

relationship. The linear mixed effects modelling used in the current study represents a more robust method for analysing data of this nature.

Lavorini *et al.* (2013) also observed significant increases in EMGpara between baseline and EOT during methacholine-induced bronchoconstriction in 44 adults with mild asthma. No specific analysis was undertaken, however, of the relationships between airways obstruction (measured using the respiratory interrupter technique) and EMGpara.

Surface EMG of the second intercostal space can detect activity in nearby muscles, and close attention to subjects' posture and upper limb movements is essential. Nearby large accessory muscles such as pectoralis major are, however, not normally recruited during tidal breathing. Postural influences on EMGpara activity have been reported (Hudson *et al.*, 2010) and we therefore ensured that subjects were well supported in the seated position to eliminate any contribution from postural activity. Subjects were also encouraged to refrain from any upper limb movements during recording periods, and EMG traces were carefully inspected to ensure absence of artefact. The EMGpara technique applied within the current study has previously been demonstrated to be reproducible (Reilly *et al.*, 2011) and to agree with values obtained simultaneously using needle electrodes (Silver & Lehr, 1981). International guidelines state surface respiratory EMG techniques to be of benefit in assessing response to altered respiratory load, and to reflect changing neural output to the respiratory muscles (American Thoracic Society & European Respiratory Society, 2002).

Although airway challenge testing does not completely mirror the acute or progressive chronic pathophysiological changes that occur in obstructive lung disease, methacholine challenge testing is thought to reproduce the adverse mechanical consequences of a moderately severe asthma exacerbation (Pride & Macklem, 2011) and therefore represents a suitable model for the interpretation of changes in pulmonary function likely to be seen in asthmatic patients. Some studies examining end-expiratory lung volume changes during acute asthma exacerbations have, however, demonstrated somewhat greater hyperinflation (mean IC values approximately 50% lower) than was observed in the current study (Pretto *et al.*, 2007; Azevedo *et al.*, 2012) and thus the potential for heterogeneity in the pathophysiological changes between these different study settings must be recognised. Further exploration of the influence of hyperinflation and airflow obstruction within clinical populations would be of interest.

This is the first study to examine the independent relative contributions of airway obstruction and hyperinflation to respiratory load and NRD. Previous cross-sectional studies have demonstrated relationships between the severity of airflow obstruction (as quantified by FEV_1), measures of end-expiratory lung volume and levels of NRD measured using EMGdi (Jolley *et al.*, 2009) and/or EMGpara (Reilly *et al.*, 2011)). These studies did not perform multivariate analyses to investigate the independent influence of each factor. In the current study, no effect of FEV_1 on the intercept of EMGpara was observed, suggesting no relationship between baseline levels of airway obstruction and NRD. This is not, however, surprising, given the narrow

range of FEV_1 values at baseline, all of which fell within normal limits. Other studies examining longitudinal changes in pulmonary function and EMGpara during acute exacerbations of lung disease (Murphy *et al.*, 2011; Reilly *et al.*, 2012) have the additional confounding influence of the metabolic load of acute illness and infection, making interpretation of relationships more challenging. These previous data show markedly larger changes in EMGpara than seen in the current study, though the populations under investigation are substantially different. It is plausible that the relationship between airflow obstruction is curvilinear, and future studies could consider examining larger cohorts to allow detailed cross-sectional or longitudinal measurements to be made across the full spectrum of disease severity.

LMM analysis did not demonstrate a significant effect of changing IC on change in EMGpara, suggesting little contribution of lung hyperinflation to overall respiratory load. The known influence of increased lung volume on the length-tension characteristics of the respiratory muscles would suggest that it is likely that hyperinflation would have an influence on levels of NRD, and indeed previous studies have demonstrated relationships between measures of end-expiratory lung volume and NRD (Jolley *et al.*, 2009; Reilly *et al.*, 2011). The severity of hyperinflation reported in these studies was, however, much more pronounced (lowest IC values of approximately 44% predicted and 1.1L respectively) than that observed in the present study, in which the median reduction in IC was only 245ml, with all participants having normal lung function at baseline. Such modest changes in end expiratory in lung volume in the current study would most likely have resulted in pulmonary mechanics remaining within the linear portion of the respiratory

system compliance curve, thus markedly greater levels of NRD and transpulmonary pressures would not have been required to achieve equivalent levels of tidal breathing. The parasternal intercostal muscles, while known to act in concert with the diaphragm (De Troyer & Estenne, 1984) and respond similarly in regard to alterations in the respiratory load-capacity balance, do not show an identical activation pattern (Reilly *et al.*, 2011). Specifically, they experience less of a decrement in their mechanical advantage with increasing lung volume than does the diaphragm (Decramer & De Troyer, 1984). Conversely, increases in operating lung volumes can have a beneficial effect under conditions of mild airflow obstruction, by increasing radial traction on the airways, increasing airway calibre (Briscoe & Dubois, 1958) and reducing airway resistance. These factors may combine to explain the lack of influence of changes in IC on EMGpara in the current study. It must also be recognised that the number of subjects within the current study somewhat limits the statistical power to interpret the relatively modest changes in lung volume elicited by the challenge test when incorporating multiple terms into the linear mixed model.

In conclusion, we have demonstrated methacholine-induced increases in neural drive to the parasternal intercostal muscles in adult subjects, the degree of which was influenced by the magnitude of airflow obstruction. Moderate changes in end-expiratory lung volume did not in this study influence levels of parasternal intercostal EMG activity.

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Competing interests

The authors declare no competing interests.

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Author contributions

VM, JM and GFR designed the study; all authors contributed to acquisition, analysis, or interpretation of data for the work. All were involved in drafting the work and/or revising it critically for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

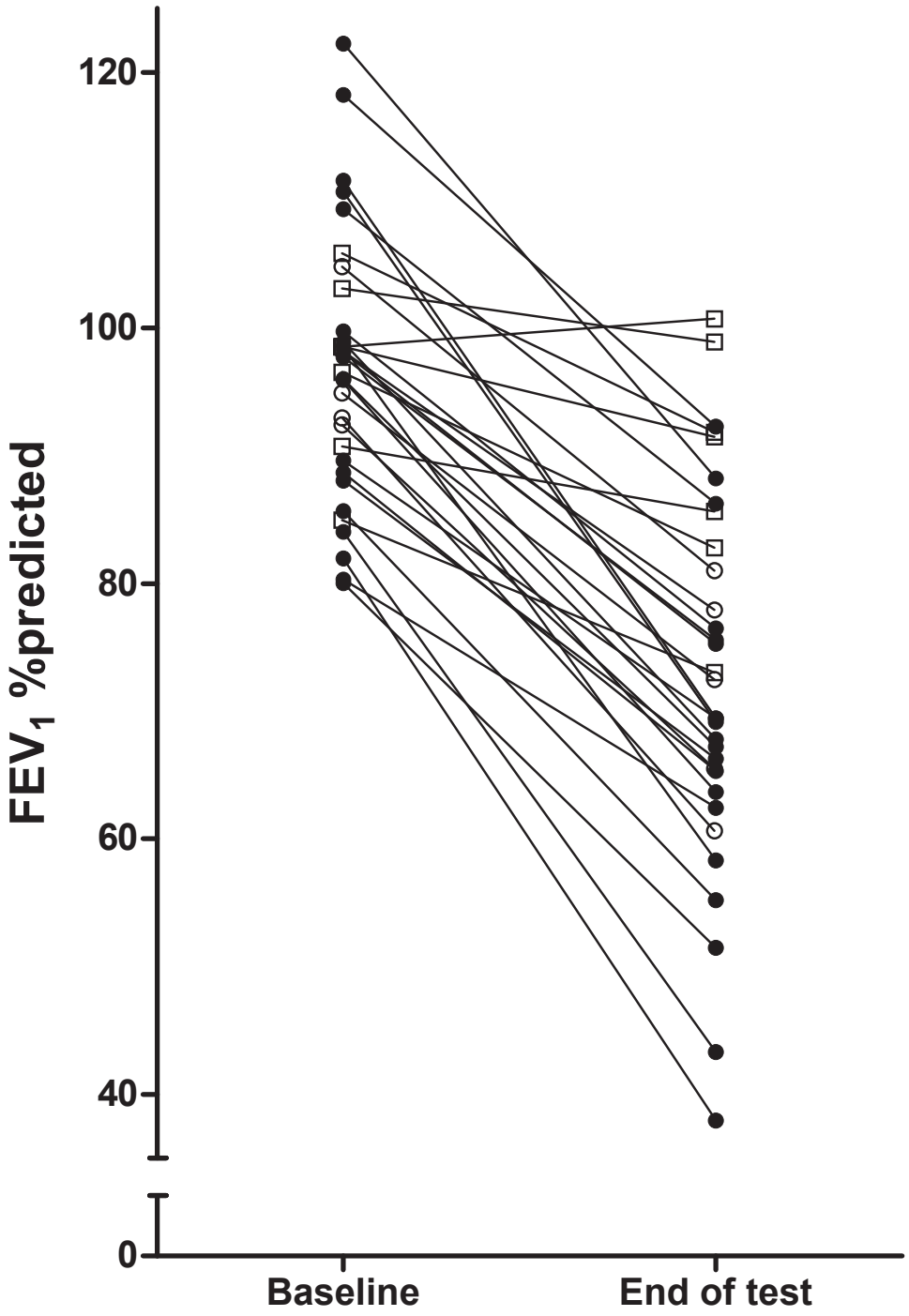
Figure legends

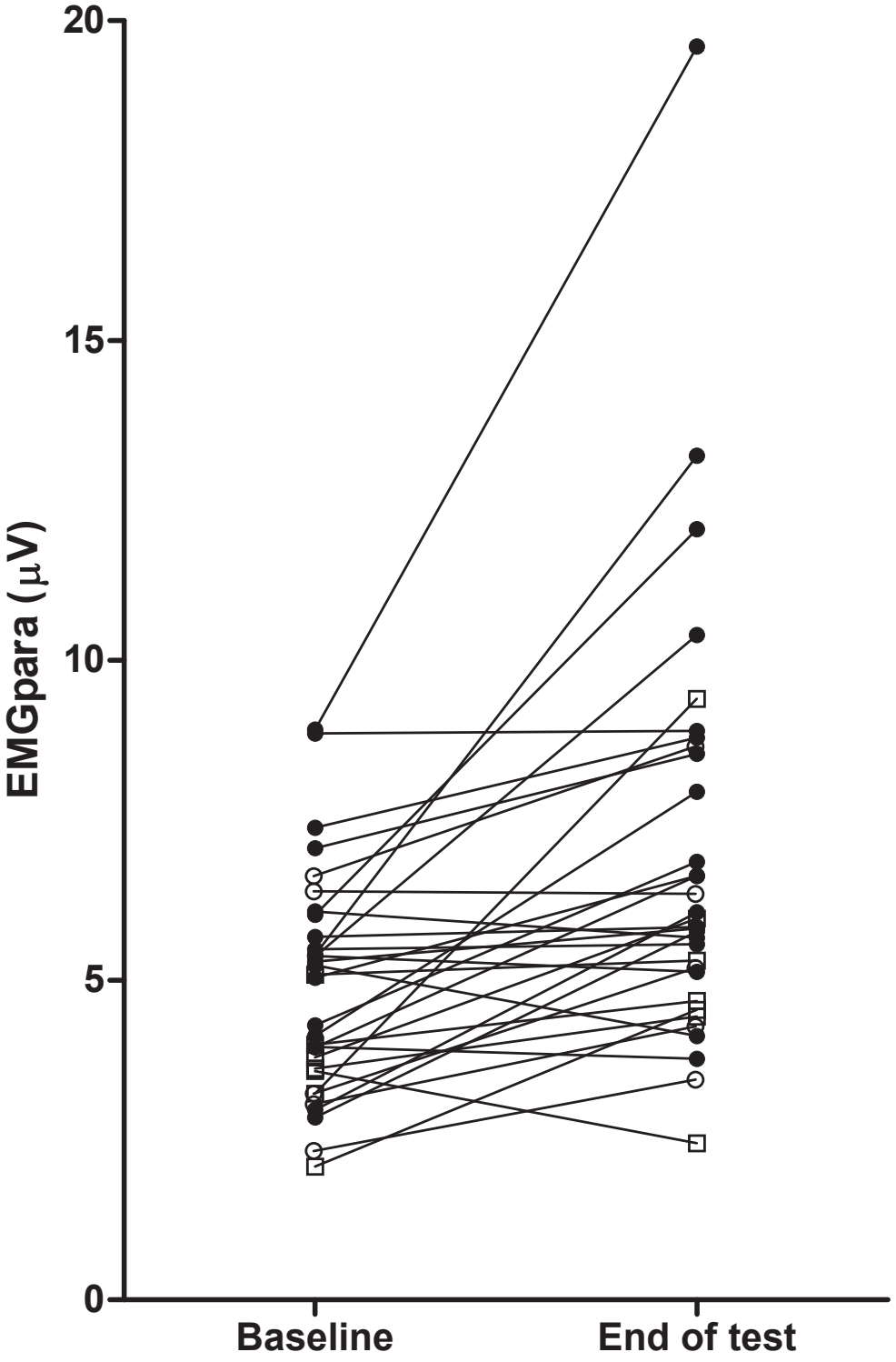
Figure 1 Baseline to end-of-test (EOT) changes in FEV_1 in 32 subjects undergoing methacholine challenge testing. Subjects undergoing measurement of IC (n=20) are indicated by filled circles, responders without IC measurements in open circles (n=5) and non-responders (n=7) by open squares.

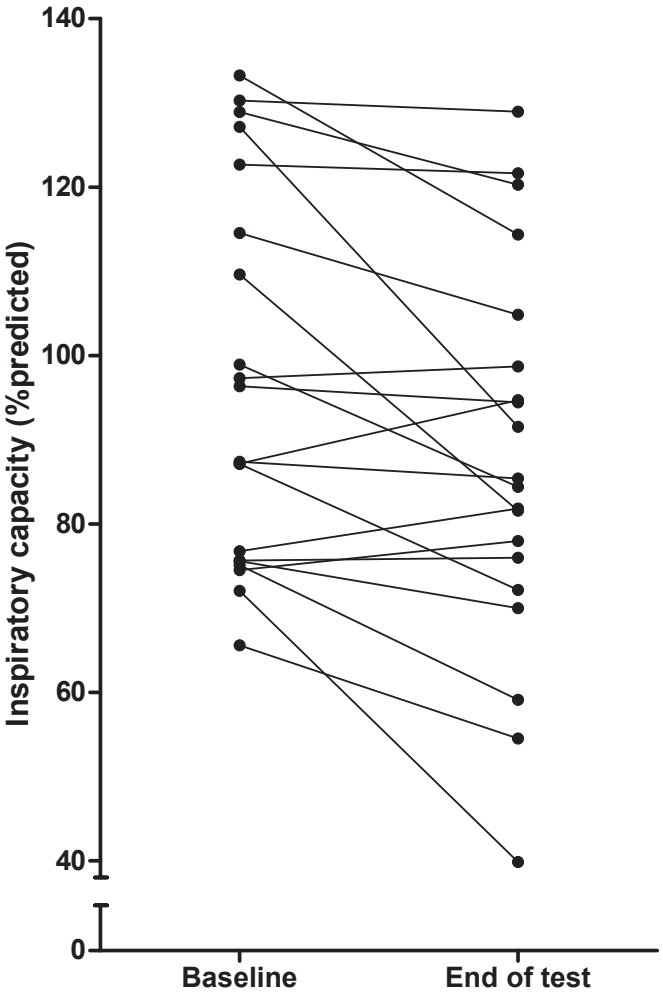
Figure 2 Baseline to end-of-test (EOT) changes in EMGpara in 32 subjects undergoing methacholine challenge testing. Subjects undergoing measurement of IC (n=20) are indicated by filled circles, responders without IC measurements in open circles (n=5) and non-responders (n=7) by open squares.

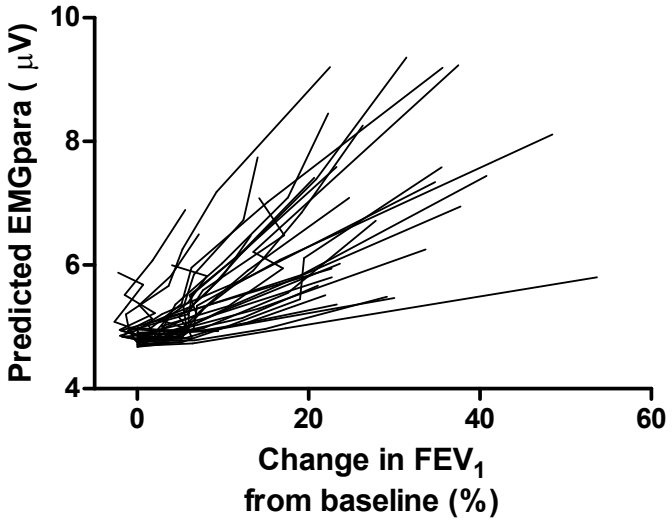
Figure 3 Baseline to end-of-test changes in inspiratory capacity in 20 subjects undergoing IC measurements during methacholine challenge testing.

Figure 4 Individual subjects' trajectories (n=32) of predicted EMGpara data based on linear mixed model analysis, plotted against measured FEV_1 change from baseline.


120
100
80
60
40
0
FEV1 %predicted
Baseline
End of test


20
15
10
5
0
EMGpara (μV)
Baseline
End of test


140
120
100
80
60
40
0
Inspiratory capacity (%predicted)
Baseline
End of test


Predicted EMGpara (μV)
10
8
6
4
0
20
40
60
Change in FEV1
from baseline (%)